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Abstract

Background: Prenatal exposure to environmental adversities, including maternal overweight/obesity, diabetes/hypertensive or mood/anxiety disorders, increases the risk for adverse neurodevelopmental outcomes in children. However, the underlying biological mechanisms remain elusive. We tested whether maternal antenatal inflammation associated with the number of neurodevelopmental delay areas in children and mediated the association between exposure to any prenatal environmental adversity and child neurodevelopmental delay.

Methods: Mother-child dyads (N=418) from the Prediction and Prevention of Preeclampsia and Intrauterine Growth Restriction (PREDO) study were followed-up to 10.8 years. We analyzed maternal plasma high-sensitivity C-reactive-protein (hsCRP) and glycoprotein acetyls at three consecutive antenatal time points, extracted data on maternal body mass index in early pregnancy, and diabetes/hypertensive disorders in pregnancy from medical records, and mood/anxiety disorders until childbirth from the Care Register for Health Care (HILMO). To estimate the number of neurodevelopmental delay areas in children across cognitive, motor and social functioning, we pooled data from HILMO on psychological development disorders with mother-reported Ages and Stages Questionnaire data on developmental milestones.

Results: Higher levels of maternal hsCRP and glycoprotein acetyls at and across all three antenatal time points were associated with 1.30 to 2.36-fold (p-values<0.02) increased relative risk for higher number of areas of child neurodevelopmental delay. Higher maternal inflammation across the three time points also mediated the effect of any prenatal environmental adversity on child neurodevelopmental delay.

Conclusions: Higher levels of maternal inflammation, especially when persisting throughout pregnancy, increase child's risk of neurodevelopmental delay and mediate the effect of prenatal environmental adversity on child neurodevelopmental delay.

Introduction

The Developmental Origins of Health and Disease (DOHaD) framework suggests that individual neurodevelopmental trajectories are shaped by environmental influences during prenatal life(1). Indeed, a number of studies have reported that prenatal exposure to environmental adversities, including maternal overweight/obesity(2-6), diabetes and hypertensive disorders(2, 7, 8), depression and anxiety(9, 10), increases child risk of neurodevelopmental delay, poorer cognition and mental disorders. As these maternal conditions associate with a quantifiable inflammatory response in pregnancy(11, 12), it has been proposed that maternal inflammation may be among the mechanisms linking prenatal environmental adversities with child neurodevelopmental outcomes(13).

Studies addressing this question have produced mixed findings. They have shown that higher maternal immunoglobulin antibodies (e.g., toxoplasma immunoglobulin antibody), C-reactive protein (CRP) or pro- and anti-inflammatory cytokines, interleukin-4 (IL-4), IL-5, IL-6, IL-8, and/or tumor necrosis factor-alpha (TNF-α) during pregnancy are associated with offspring neurodevelopmental delay(14), impaired neurocognitive development(15-18), internalizing and externalizing problems(19), autism spectrum disorders (ASD)(14, 20, 21) and schizophrenia(22-26). According to recent neuroimaging studies, higher maternal IL-6 during pregnancy is also associated with alterations in newborn structural and functional brain connectivity and development in infancy across various brain regions and networks, and these alterations may mediate the associations between IL-6 and lower impulse control, poorer working memory and cognition in children(16-18). However, some studies have reported that higher levels of these maternal inflammatory biomarkers are associated with higher cognitive abilities, lower risk of neurological abnormalities(15, 27, 28), lower risk of schizophrenia(25), major depression(29), and ASD(30) in the children, and some others have reported that these maternal inflammatory biomarkers are not associated with risk of ASD(23), schizophrenia(31) or bipolar disorder(32, 33) in children.

While the mixed findings may result from methodological differences between the studies, including variation in the studied set of inflammatory biomarkers and small sample sizes, they may also reflect reliance on a single measurement point(14, 21, 22, 26, 28, 34, 35) and variation in the stage of pregnancy when inflammatory biomarkers were measured. This is critical as pregnancy triggers a marked inflammatory response showing large inter-individual differences(36). Hence, it remains unknown if there exists a critical window of vulnerability during pregnancy, and if fluctuations in maternal inflammatory biomarkers during pregnancy or persistently high levels are more critical for *in utero* neurodevelopment than the level of inflammation at one single point during pregnancy. To the best of our knowledge, previous studies have not addressed these possibilities even if inflammatory biomarkers were measured more than once during pregnancy(16-18, 20, 29, 31-33).

Furthermore, we are not aware of any studies testing if maternal inflammatory biomarkers mediate the associations between prenatal environmental adversities and child neurodevelopment. One study has demonstrated that lower maternal IL-8, but not maternal IL-β1, IL-6, IL-10 or TNF-α, measured at 32 gestational weeks, mediated the association between maternal socioeconomic disadvantage during pregnancy and higher risk of child neurological abnormalities(27).

We tested if maternal high-sensitivity CRP (hsCRP) and glycoprotein acetyls, measured at three consecutive antenatal timepoints, were associated with higher risk of neurodevelopmental delay in their children. We also tested if the predictive significance of hsCRP and glycoprotein acetyls varied according to the stage of pregnancy and if any fluctuations or persistently high levels in these inflammatory biomarkers predicted child risk of neurodevelopmental delay. Furthermore, we tested if maternal hsCRP and glycoprotein acetyls mediated the associations between exposure to any prenatal environmental adversity (maternal overweight/obesity, diabetes, hypertensive, mood and/or anxiety disorders diagnosed before and during pregnancy) and child neurodevelopmental delay and if these associations varied by child sex, since there is evidence that the associations between

prenatal environmental adversities and child neurodevelopmental outcomes may be sex-specific(37, 38). We focused on hsCRP and glycoprotein acetyls since both of these inflammatory biomarkers have long half-lives, indicate systemic, low-grade chronic inflammation(39) and are elevated in women during pregnancy(40). HsCRP is widely used in clinical practice and is among the most extensively used inflammatory biomarkers in research. High hsCRP is linked with depression(41, 42) and cardiovascular mortality(43). Glycoprotein acetyls is a novel systemic inflammatory biomarker. It represents a composite signal of changes in multiple circulating glycoproteins and is suggestive of a prolonged low-grade inflammatory state(39). Both hsCRP and glycoprotein have been linked with overweight/obesity, chronic hypertension, preeclampsia, gestational diabetes and depression during pregnancy(44).

Methods and Materials

Sample

The participants come from the Prediction and Prevention of Pre-eclampsia and Intrauterine Growth Restriction (PREDO) study(45). We enrolled 1,079 pregnant women to the clinical subsample: 969 had one or more, and 110 had none of the known risk factors for pre-eclampsia and intrauterine growth restriction (IUGR). The women were recruited when they arrived for their first ultrasound screening at 12-14 gestational weeks from 10 hospitals in Southern and Eastern Finland.

Of the 1,079 women, 420 donated blood at up to three antenatal time points. Economic constraints restricted blood sampling to the three largest study hospitals. The samples were taken at median (interquartile range) 13.0(12.6-13.4), 19.3(19.0-19.7), and 27.0(26.6-27.6) gestational weeks. Of the 420 women, 379(90.2%) provided blood samples at all three consecutive timepoints, 39(9.3%) at two timepoints, and 2(0.5%) at one timepoint. Of these women, 418(99.5%) had data available from Care Register for Health Care (HILMO) on child psychological development disorder diagnoses up

to child age of 7.1-10.8 years. These data were enriched by mother-reports of child developmental milestones available for 195(46.7% of the 418 women with blood samples and child diagnoses data) children at 2.3-5.7 years of age.

Women providing blood samples were younger (32.6 vs. 33.5 years, p=0.007) and less likely to be obese (body mass index [BMI]>30 kg/m^2) (29.4% vs. 39.3%, p=0.003) than women who did not. The groups with and without blood samples did not differ in other study variables and those with and without mother-reports on child developmental milestones did not differ in any study variables (p>0.06).

All participants signed informed consents. The PREDO study protocol was approved by ethics committees of the Helsinki and Uusimaa Hospital District and aligns with The Code of Ethics of the World Medical Association (Declaration of Helsinki).

Maternal inflammatory biomarkers

Venous blood samples were drawn from antecubital vein between 7-9 AM, after at least a 10 hour fast with plasma separated immediately and stored at -80°C.HsCRP (mg/L) was analyzed with a Beckman-Coulter CRP immunoturbidometric assay and Olympus AU680 analyzer (Beckman Coulter Inc., CA, USA)(intra-assay variation was between 2.6 % (n=10, mean 1.20 mg/L) and 0.7 % (n=10, mean 65 mg/L) and inter-assay variations were 3.5 % (n=30, mean 1.07 mgLl), 1.2 % (n=30, mean 11.5 mg/L) and 2.9% (n=30, mean 73 mg/L). The limit of detection of the hsCRP method is 0.02 mg/L and the functional sensitivity was better than 0.1 mg/L. Glycoprotein acetyls (mmol/L) were analyzed using a high-throughput nuclear magnetic resonance (NMR) metabolomics platform (1HNMR spectra, Nightingale Ltd.; Espoo, Finland)(46).

Child neurodevelopmental delay

Psychological development disorders were identified from HILMO since birth until 31 December 2016. HILMO includes all in-patient (since 1969) and outpatient treatments (since 1998) by

physicians in public specialized-care, and covers psychiatric diagnoses well (47). Diagnoses are based on the International Classification of Diseases, Tenth Revision (ICD-10), identifying developmental disorders of speech and language (F80), scholastic skills (F81) and motor function (F82), mixed specific developmental disorders (F83), and ASD (F84).

In addition to the diagnoses, the children were considered to display neurodevelopmental delay if their score on The Ages and Stages Questionnaires (ASQ) Third edition was ≤-2SD for age(48). ASQ measures communication, problem solving, gross motor, fine motor and personal/social skills and comprises 30 age-appropriate items scored "10" if the child can master the skill, "5" if the skill is emerging/occasional, and "0" if the child cannot perform the skill(48-50). Age-appropriate ASQ means and SDs were derived from the reference population of the children participating in the PREDO study (2). The groups comprised children aged 25.5-28.5 (n=2), 28.5-31.5 (n=21), 31.5-34.5 (n=27), 34.5-39.0 (n=23), 39.0-45.0 (n=24), 45.0-51.0 (n=33), 51.0-57.0 (n=30), and 57.0-66.0 (n= 24) months. We allowed 30% deviation from the questionnaire-specific age translating into 3 weeks in the youngest and 2.7 months in the oldest age group.

The ASQ (≤-2SD for age on any ASQ domain) correctly identified psychological development disorder status for 85.7% (430/502; 502 of 1079 children in clinical subsample had ASQ data; chance-corrected first-order agreement coefficient [AC1](51)=0.82, 95%Confidence Interval [95%CI](0.78, 0.86) and 88.7% (173/195; 195 of the 418 children in our analytic sample had ASQ data; AC1=0.85, 95%CI (0.79, 0.92) of children in the clinical and analytics subsample of the current study, respectively (Supplemental Figure 1). Any delay in ASQ also increased significantly the odds (odds ratio=[OR] 9.8, 95% CI 4.4, 21.4, p<0.0001, OR=11.5, 95% Confidence Interval [95% CI] 2.8, 46.6, p=0.0007) of any psychological development disorder diagnoses (F80-F84) in the clinical and the analytic subsample of the current study, respectively.

Using information from both HILMO and ASQ, and to increase statistical power, we created one broad outcome variable based on the number of areas in which the child displayed neurodevelopmental delay: (1) delay in cognitive development (F80, F81, F83 or scoring ≤-2SD for age on ASQ communication and/or problem solving skills; none of the children with F83 had specific motor development disorders; (2) delay in motor development (F82 or scoring ≤-2SD for age on ASQ fine motor and/or gross motor skills), (3) delay in social development (F84 or scoring ≤-2SD for age on ASQ personal/social skills). Hence, this variable captures delay in all three (3), delay in any two (2), delay in any one (1) and no delay in any area (0).

Prenatal environmental adversity

These data were derived from the Medical Birth Register (MBR), HILMO and medical records. Maternal diabetes and hypertensive disorders were extracted from the medical records and verified by an expert jury comprising two medical doctors and a research nurse with expertise in obstetrics and gynecology.

Maternal early pregnancy overweight/obesity was defined as BMI ≥25 kg/m^2 verified by measurement conducted at first antenatal clinic visit at a mean 8.5 (SD=1.5) gestational weeks. Diabetes included type 1 diabetes and gestational diabetes (fasting, 1 or 2 h plasma glucose during a 75 g oral glucose tolerance test ≥5.1, 10.0, or 8.5 mmol/L that emerged or was first identified during pregnancy). Hypertensive disorders included chronic hypertension (systolic/diastolic blood pressure ≥140/90 mmHg present pre-pregnancy or diagnosed before 20th week of gestation, gestational hypertension (systolic/diastolic blood pressure ≥140/90 mmHg on ≥2 occasions at least 4 h apart in a woman who was normotensive before 20th week of gestation) and preeclampsia (systolic/diastolic blood pressure ≥140/90 mmHg on ≥2 occasions at least 4 h apart in a women who was normotensive before 20th week of gestation with proteinuria ≥300 mg/24 h). Maternal mood (ICD-10 since 1996: F3; Diagnostic and Statistical Manual for Mental Disorders, Third Revision [DSM-

III-R] between 1987-1995: 296, 3004A, 3011D) and anxiety/neurotic disorder (ICD-10 since 1996: F4; DSM-III-R: 3000-3003, 3006-3009, 3078A, 309) diagnoses were identified since 1987 until childbirth.

Because of high comorbidity of these maternal conditions (Supplemental Table 1) we combined them into one composite variable of any prenatal environmental adversity.

Covariates and moderators

Based on previous literature, the following variables were included as covariates: maternal age at childbirth (<40≥ years)(44), smoking during pregnancy (yes/no)(52), occupational status (manual laborer/junior or senior clerical/entrepreneur)(53, 54), education (basic/secondary vs. tertiary)(44) and alcohol use (yes/no)(55). Child sex (girl/boys) and year of birth were used both as covariates and moderators, because the associations between prenatal environmental adversities and child neurodevelopmental outcomes may be sex-specific(37, 38) and because of heterogeneity in follow-up time. All covariates/moderators were derived from MBR except for maternal education and alcohol use, which were reported in early pregnancy.

Statistical analyses

Poisson regression tested if the relative risk (RR) for higher number of areas of neurodevelopmental delay was increased for children of women with higher levels of hsCRP and glycoprotein acetyls at the three sampling points.

To test whether fluctuating or persistently elevated levels of maternal hsCRP and glycoprotein acetyls were associated with the number of areas of child neurodevelopmental delay, we first applied latent class analysis (LCA) to identify subgroups of women based on their antenatal inflammation profiles defined by both inflammatory biomarkers simultaneously. We compared two to six subgroups solutions using the following criteria for the most optimal solution(56): (1) Akaike Information Criterion, Bayesian Information Criterion of goodness-of-fit, (2) reasonable

distribution of participants across subgroups (at least 10% of the sample), (3) high certainty of classification identified by posterior probabilities, and (4) clinical relevance. By using Poisson regression, we then examined if the number of areas of neurodevelopmental delay varied according to the maternal inflammation subgroups identified by the LCA.

We explored whether maternal antenatal inflammation mediated the association between prenatal environmental adversity (maternal overweight/obesity, diabetes, hypertensive disorders, mood or anxiety disorders) and the number of child neurodevelopmental delay areas using the SPSS PROCESS macro with 5000 bootstrapped samples(57). As a mediator we used the inflammation subgroups identified by the LCA. Before testing mediation, we assured that the criteria for mediation were met: namely, that the predictor, mediator and outcome variables were interrelated.

We made adjustments for all covariates, and tested moderation by child birth year and sex by introducing interaction terms of maternal antenatal inflammation x child birth year and maternal antenatal inflammation x child sex into the regression models. We also conducted sensitivity analyses assuring that the following conditions did not affect our findings: we excluded women diagnosed for the first time with mood (n=20) and/or anxiety/neurotic disorder (n=18) after childbirth, women diagnosed with acute infectious diseases within a month of any of the blood sampling points (n=13; these infections were identified with previously described ICD-10 diagnostic codes)(58, 59), herpes simplex (n=3; A60 and B00), (none of the women had toxoplasmosis), rheumatoid arthritis (n=2; M05, M06, M12, M45) or asthma (n=11; J45, J46) with diagnoses derived from HILMO. We also excluded women whose maximum hemoglobin value during pregnancy was < 105 g/L (n= 79)(60), as indicated by the patient case reports. Finally, we examined the associations with the number of areas of neurodevelopmental delay where we defined neurodevelopmental delay only based on HILMO data to make sure that mother-reports did not bias the results.

We normalized hsCRP with natural logarithm transformation and standardized hsCRP and glycoprotein acetyls (Mean=0, SD=1) to facilitate interpretation. RR and 95% CI show effect sizes; in the mediation analyses effect sizes are presented as regression coefficients and RR.

Poisson regression was performed using SAS 9.4 (SAS Institute, Inc., Cary, NC, USA); mediation analysis using SPSS-IBM (Software, v.24.0 SPSS); latent class analyses using Stata 15 (StataCorp. 2017. Stata Statistical Software: Release 15. College Station, TX: StataCorp LLC).

Results

Sample characteristics are in Table 1. HsCRP and glycoprotein acetyls were intercorrelated (Pearson r's≥0.34, p<0.001) showing high rank-order stability across pregnancy (intraclass correlations≥0.76 and ≥0.72 for hsCRP and glycoprotein acetyls, respectively).

Maternal inflammatory biomarkers at the three antenatal sampling points

Table 2 shows that across all antenatal sampling points, higher maternal hsCRP and glycoprotein acetyl levels were associated with increased RR for higher number of child neurodevelopmental delay areas. These associations remained when adjusted for all covariates and prenatal environmental adversities, except for the associations with glycoprotein acetyls at the first and second blood sampling points, which were no longer significant when adjusted for prenatal environmental adversities (Table 2). Supplemental Table 2 shows the associations with the three specific neurodevelopmental areas as outcomes.

Fluctuating and persistently high levels of maternal inflammatory biomarkers

Figure 1 shows the most optimal LCA solution identified three subgroups of women who differed from each other in their hsCRP and glycoprotein acetyl levels. The inflammatory biomarkers showed high stability and lack of fluctuation across pregnancy in each group. The groups showed

consistently low (n=71[17%]), moderate (n=181[43.3%]) and high (n=166[39.7%]) levels of both hsCRP and glycoprotein acetyls across pregnancy. Supplemental Table 3 presents the LCA selection criteria for the 2-6 subgroups solutions.

Child risk of higher number of neurodevelopmental delay areas increased linearly across maternal groups with low, moderate and high levels of antenatal inflammation (RR=2.36, 95% CI 1.57, 3.55, p<0.001). These associations remained when adjusted for covariates and prenatal environmental adversities (p-values <0.002).

We also compared if glycoprotein acetyls added to the risk prediction of child neurodevelopmental delay in comparison to hsCRP alone. Before proceeding to this comparison, we identified the most optimal LCA solution based on hsCRP alone. LCA identified three groups of women with consistently low, moderate and high hsCRP levels (Supplemental Table 4). Child risk for higher number of neurodevelopmental delay areas increased significantly according to maternal hsCRP groups (RR=1.83, 95% CI 1.24, 2.70, p<0.001). Using Likelihood-Ratio-Test, we then compared goodness-of-fit of the following two nested models: the first, full model included the three-group LCA solution based on hsCRP and glycoprotein acetyls and the three-group LCA solution based on hsCRP, and the second, restricted model, included the three-group LCA solution based on hsCRP alone. Goodness-of-fit of the full model was better (deviance=278.39) than the restricted model (deviance=288.50) (Likelihood-Ratio-Test p<0.001).

Moderation and mediation analyses

None of the associations were moderated by child sex or birth year (p-values > 0.06; data not shown).

Figure 2 shows, first, that criteria for testing mediation were met, and second, that higher level of maternal antenatal inflammation partially mediated the effect of any prenatal environmental

adversity on the number of areas of child neurodevelopmental delay (the effect size proportion mediated=55.2%, 95% CI 18.7-95.5).

Sensitivity analyses

Supplemental Table 5 shows that the exclusion of women diagnosed for the first time with any mood or anxiety disorder after childbirth, the exclusion of women with acute infections, herpes simplex, rheumatoid arthritis, asthma or anemia or restricting the analyses to child HILMO diagnoses only did not substantially change the associations.

Discussion

In this prospective study, higher levels of maternal antenatal hsCRP and glycoprotein acetyls were associated with higher number of child neurodevelopmental delay areas in a follow-up to 10.8 years. The number of neurodevelopmental delay areas was the highest in children of women whose inflammation levels of both of these inflammatory biomarkers and across the three consecutive antenatal sampling points were the highest. Of note is that while the levels of hsCRP and glycoprotein acetyls are increased during pregnancy(40), which was also observed in our sample, we did not observe fluctuations in the women's inflammatory response. Instead, inflammation showed a high degree of stability. Associations between maternal antenatal inflammation and the number of neurodevelopmental delay areas were similar in girls and boys and not driven by acute infections or other proinflammatory conditions, as the associations did not substantially change when we excluded women who had an acute infection within a month of the blood sampling, women with herpes simplex, rheumatoid arthritis, asthma or anemia during pregnancy. Hence, our findings show that persistently high levels of maternal antenatal inflammation may be critical for *in utero* neurodevelopment.

Our findings also suggest that persistently high levels of antenatal inflammation partially mediated the link between exposure to any prenatal environmental adversity, namely to maternal

overweight/obesity, diabetes, hypertensive, mood and anxiety disorders, and child neurodevelopmental delay. Having any of these adversities was associated with an over 2-fold increased risk of higher number of areas of child neurodevelopmental delay and was also significantly associated with persistently high levels of antenatal inflammation. The mediation finding suggests that maternal antenatal inflammation may provide a target for therapeutic interventions preventing the child from deviations from his/her optimal neurodevelopmental trajectory, such as dietary interventions employing vitamin D, probiotics and polyunsaturated fatty acids(61-64). They have been shown to be associated with reduced inflammation and they may also provide neurodevelopmental benefits for the fetus and mental health benefits for the pregnant women(65-70).

Our findings are in line with most of the previous studies(14-18, 20) showing associations of maternal antenatal inflammation with impaired neurodevelopment, cognition, ASD and altered brain structural and functional connectivity and development in children. These studies have, however, been based on small sample sizes and relied mostly on a single blood sample for the measurement of inflammatory biomarkers at gestational stages varying across the studies. However, the current and previous studies are not directly comparable, as the studied set of inflammatory biomarkers differs. Many of the previous studies have used very rapidly degrading inflammatory markers, most commonly IL-6, which we lacked in this study. Instead, we focused on hsCRP, which is an acute-phase protein with a longer half-life than IL-6(71) and on glycoprotein acetyls which shows even slower kinetics than hsCRP and which has not been studied before in this context. These biomarkers allowed a stable estimation of the participants' inflammatory state across pregnancy(39), and as they increase in response to pregnancy(40), they capture antenatal inflammation. In our study, glycoprotein acetyls also improved the prediction of child neurodevelopmental delay over hsCRP alone, suggesting that child neurodevelopmental delay may be best predicted by their combination. Whether the prediction could be further improved by

including into our model other inflammatory and related biomarkers, such as maternal steroid hormones, which we concurrently do not have, and which is a study limitation, warrants further study. Our outcome, representing an aggregate measure of neurodevelopmental delay to indicate deviation from typical neurodevelopmental trajectory rather than capturing specific neurodevelopmental deficiencies, has not been studied previously either. Shifts and/or disruptions from the typical neurodevelopmental trajectory in childhood often precede later development of severe mental disorders(72-75), suggesting that the outcomes may lie on a same continuum of neurodevelopmental adversities.

Our results are in agreement with the evidence from studies examining neurodevelopmental outcomes in relation to maternal antenatal infection(76-78). Interestingly, a recent study among 1,791,520 Swedish children found that fetal exposure to maternal infection increased the risk for ASD in the offspring, but did not find differences between severity of infection and the risk of ASD(76), suggesting that this is not a dose-response relationship, but that the presence of infection *per se* may play a role in neurodevelopmental programming.

Molecular and cellular mechanisms that may underlie the neurodevelopmental programming of the offspring brain remain elusive(79). Animal studies have shown that maternal inflammation increases microglial density and/or activation in the fetal brain(79), driving these cells from physiological to more pathological roles, both short-term and persistently(80); in humans aberrant microglia have been identified postmortem in schizophrenia, depression, Tourette syndrome, and ASD(81). Also, animal studies have shown that exposure to maternal inflammation may result in over- and undergrowth of brain volume, both changes associated with ASD and schizophrenia(79). Alterations in synapse formation and dendritic development are core aspects of psychiatric pathophysiology that may mechanistically link prenatal inflammation to psychiatric risk. Animal studies have shown that prenatal exposure to maternal inflammation persistently reduced cortical

dendritic spine density, turnover, and connectivity, as well as altered excitatory and inhibitory synaptic transmission(82).

Our study limitation is that 75.4% of our study participants were recruited based on their high risk factor status for pre-eclampsia and IUGR, which may have contributed to the relatively high comorbidity of prenatal environmental adversities in our sample. This limits the generalizability of our findings to general populations of pregnant women and hinders studying the unique effects of any individual environmental adversity. The high risk is, however, also a strength of our study. It resulted in a high prevalence of prenatal environmental adversities in our sample, providing increased statistical power to examine the effects of inflammation and the mediation of prenatal environmental adversities via inflammation on child neurodevelopment. Another strength of our study includes the prospective study design, availability of data from the MBR, HILMO and medical records which resulted in nearly null follow-up attrition. Attrition was, however, notable with regard to mother-reported child neurodevelopmental delay. As a result, we may have missed detecting children with milder delays in neurodevelopment. We believe that missing such cases may have rather decreased than increased our ability to detect significant associations. Finally, measuring maternal inflammation at three antenatal time points may still not be sufficient enough to capture short-lived fluctuations in antenatal inflammation.

In conclusion, our study showed that persistently high levels of maternal antenatal inflammation, across three antenatal sampling points, were associated with, and mediated the effects of prenatal environmental adversities on the number of areas of child neurodevelopmental delay. Our findings provide support for the hypothesis that maternal inflammation may represent one mechanism underlying the neurodevelopmental programming of offspring brain.

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References

1. Barker DJ. The origins of the developmental origins theory. Journal of internal medicine. 2007;261(5):412-7.

2. Girchenko P, Tuovinen S, Lahti-Pulkkinen M, Lahti J, Savolainen K, Heinonen K, et al. Maternal early pregnancy obesity and related pregnancy and pre-pregnancy disorders: associations with child developmental milestones in the prospective PREDO Study. International journal of obesity (2005). 2018.
3. Pugh SJ, Richardson GA, Hutcheon JA, Himes KP, Brooks MM, Day NL, et al. Maternal Obesity and Excessive Gestational Weight Gain Are Associated with Components of Child Cognition. The Journal of nutrition. 2015;145(11):2562-9.
4. Huang L, Yu X, Keim S, Li L, Zhang L, Zhang J. Maternal prepregnancy obesity and child neurodevelopment in the Collaborative Perinatal Project. International journal of epidemiology. 2014;43(3):783-92.
5. Rodriguez A. Maternal pre-pregnancy obesity and risk for inattention and negative emotionality in children. Journal of child psychology and psychiatry, and allied disciplines. 2010;51(2):134-43.
6. Yeung EH, Sundaram R, Ghassabian A, Xie Y, Buck Louis G. Parental Obesity and Early Childhood Development. Pediatrics. 2017;139(2).
7. Fraser A, Nelson SM, Macdonald-Wallis C, Lawlor DA. Associations of existing diabetes, gestational diabetes, and glycosuria with offspring IQ and educational attainment: the Avon Longitudinal Study of Parents and Children. Experimental diabetes research. 2012;2012:963735.
8. Ghassabian A, Sundaram R, Wylie A, Bell E, Bello SC, Yeung E. Maternal medical conditions during pregnancy and gross motor development up to age 24 months in the Upstate KIDS study. Developmental medicine and child neurology. 2016;58(7):728-34.
9. Glover V, O'Donnell KJ, O'Connor TG, Fisher J. Prenatal maternal stress, fetal programming, and mechanisms underlying later psychopathology-A global perspective. Development and psychopathology. 2018;30(3):843-54.
10. Tuovinen S, Lahti-Pulkkinen M, Girchenko P, Lipsanen J, Lahti J, Heinonen K, et al. Maternal depressive symptoms during and after pregnancy and child developmental milestones. Depression and anxiety. 2018;35(8):732-41.
11. Basu S, Haghiac M, Surace P, Challier JC, Guerre-Millo M, Singh K, et al. Pregravid obesity associates with increased maternal endotoxemia and metabolic inflammation. Obesity (Silver Spring, Md). 2011;19(3):476-82.
12. Pantham P, Aye IL, Powell TL. Inflammation in maternal obesity and gestational diabetes mellitus. Placenta. 2015;36(7):709-15.
13. Hantsoo L, Kornfield S, Anguera MC, Epperson CN. Inflammation: A Proposed Intermediary Between Maternal Stress and Offspring Neuropsychiatric Risk. Biological psychiatry. 2019;85(2):97-106.
14. Goines PE, Croen LA, Braunschweig D, Yoshida CK, Grether J, Hansen R, et al. Increased midgestational IFN-gamma, IL-4 and IL-5 in women bearing a child with autism: A case-control study. Molecular autism. 2011;2:13.
15. Ghassabian A, Albert PS, Hornig M, Yeung E, Cherkerzian S, Goldstein RB, et al. Gestational cytokine concentrations and neurocognitive development at 7 years. Translational psychiatry. 2018;8(1):64.
16. Rudolph MD, Graham AM, Feczko E, Miranda-Dominguez O, Rasmussen JM, Nardos R, et al. Maternal IL-6 during pregnancy can be estimated from newborn brain connectivity and predicts future working memory in offspring. Nature neuroscience. 2018;21(5):765-72.
17. Rasmussen JM, Graham AM, Entringer S, Gilmore JH, Styner M, Fair DA, et al. Maternal Interleukin-6 concentration during pregnancy is associated with variation in frontolimbic white matter and cognitive development in early life. NeuroImage. 2019;185:825-35.
18. Graham AM, Rasmussen JM, Rudolph MD, Heim CM, Gilmore JH, Styner M, et al. Maternal Systemic Interleukin-6 During Pregnancy Is Associated With Newborn Amygdala Phenotypes and Subsequent Behavior at 2 Years of Age. Biological psychiatry. 2018;83(2):109-19.
19. Mac Giollabhui N, Breen EC, Murphy SK, Maxwell SD, Cohn BA, Krigbaum NY, et al. Maternal inflammation during pregnancy and offspring psychiatric symptoms in childhood: Timing and sex matter. Journal of psychiatric research. 2019;111:96-103.

20. Brown AS, Sourander A, Hinkka-Yli-Salomaki S, McKeague IW, Sundvall J, Surcel HM. Elevated maternal C-reactive protein and autism in a national birth cohort. Molecular psychiatry. 2014;19(2):259-64.
21. Chen J, Xin K, Wei J, Zhang K, Xiao H. Lower maternal serum 25(OH) D in first trimester associated with higher autism risk in Chinese offspring. J Psychosom Res. 2016;89:98-101.
22. Buka SL, Tsuang MT, Torrey EF, Klebanoff MA, Bernstein D, Yolken RH. Maternal infections and subsequent psychosis among offspring. Archives of general psychiatry. 2001;58(11):1032-7.
23. Zhang J, Luo W, Huang P, Peng L, Huang Q. Maternal C-reactive protein and cytokine levels during pregnancy and the risk of selected neuropsychiatric disorders in offspring: A systematic review and meta-analysis. Journal of psychiatric research. 2018;105:86-94.
24. Brown AS, Patterson PH. Maternal infection and schizophrenia: implications for prevention. Schizophrenia bulletin. 2011;37(2):284-90.
25. Goldstein JM, Cherkerzian S, Seidman LJ, Donatelli JAL, Remington AG, Tsuang MT, et al. Prenatal maternal immune disruption and sex-dependent risk for psychoses. Psychological medicine. 2014;44(15):3249-61.
26. Canetta S, Sourander A, Surcel HM, Hinkka-Yli-Salomaki S, Leiviska J, Kellendonk C, et al. Elevated maternal C-reactive protein and increased risk of schizophrenia in a national birth cohort. The American journal of psychiatry. 2014;171(9):960-8.
27. Gilman SE, Hornig M, Ghassabian A, Hahn J, Cherkerzian S, Albert PS, et al. Socioeconomic disadvantage, gestational immune activity, and neurodevelopment in early childhood. Proceedings of the National Academy of Sciences of the United States of America. 2017;114(26):6728-33.
28. Spann MN, Monk C, Scheinost D, Peterson BS. Maternal Immune Activation During the Third Trimester Is Associated with Neonatal Functional Connectivity of the Salience Network and Fetal to Toddler Behavior. The Journal of neuroscience : the official journal of the Society for Neuroscience. 2018;38(11):2877-86.
29. Gilman SE, Cherkerzian S, Buka SL, Hahn J, Hornig M, Goldstein JM. Prenatal immune programming of the sex-dependent risk for major depression. Translational psychiatry. 2016;6(5):e822.
30. Zerbo O, Traglia M, Yoshida C, Heuer LS, Ashwood P, Delorenze GN, et al. Maternal mid-pregnancy C-reactive protein and risk of autism spectrum disorders: the early markers for autism study. Translational psychiatry. 2016;6:e783.
31. Ellman LM, Deicken RF, Vinogradov S, Kremen WS, Poole JH, Kern DM, et al. Structural brain alterations in schizophrenia following fetal exposure to the inflammatory cytokine interleukin-8. Schizophrenia research. 2010;121(1-3):46-54.
32. Chudal R, Sourander A, Surcel HM, Sucksdorff D, Hinkka-Yli-Salomaki S, Brown AS. Gestational maternal C--reactive protein and risk of bipolar disorder among young individuals in a Nationwide Birth Cohort. Journal of affective disorders. 2017;208:41-6.
33. Cheslack-Postava K, Cremers S, Bao Y, Shen L, Schaefer CA, Brown AS. Maternal serum cytokine levels and risk of bipolar disorder. Brain, behavior, and immunity. 2017;63:108-14.
34. Jones KL, Croen LA, Yoshida CK, Heuer L, Hansen R, Zerbo O, et al. Autism with intellectual disability is associated with increased levels of maternal cytokines and chemokines during gestation. Molecular psychiatry. 2017;22(2):273-9.
35. Goldstein JM, Holsen L, Huang G, Hammond BD, James-Todd T, Cherkerzian S, et al. Prenatal stress-immune programming of sex differences in comorbidity of depression and obesity/metabolic syndrome. Dialogues in clinical neuroscience. 2016;18(4):425-36.
36. Wang Q, Wurtz P, Auro K, Makinen VP, Kangas AJ, Soininen P, et al. Metabolic profiling of pregnancy: cross-sectional and longitudinal evidence. BMC medicine. 2016;14(1):205.
37. Buss C, Davis EP, Shahbaba B, Pruessner JC, Head K, Sandman CA. Maternal cortisol over the course of pregnancy and subsequent child amygdala and hippocampus volumes and affective problems. 2012;109(20):E1312-E9.
38. Suarez A, Lahti J, Czamara D, Lahti-Pulkkinen M, Knight AK, Girchenko P, et al. The Epigenetic Clock at Birth: Associations With Maternal Antenatal Depression and Child Psychiatric Problems. Journal of the American Academy of Child and Adolescent Psychiatry. 2018;57(5):321-8.e2.

39. Ritchie Scott C, Würtz P, Nath Artika P, Abraham G, Havulinna Aki S, Fearnley Liam G, et al. The Biomarker GlycA Is Associated with Chronic Inflammation and Predicts Long-Term Risk of Severe Infection. Cell Systems. 2015;1(4):293-301.
40. Wang Q, Würtz P, Auro K, Mäkinen V-P, Kangas AJ, Soininen P, et al. Metabolic profiling of pregnancy: cross-sectional and longitudinal evidence. BMC medicine. 2016;14(1):205-.
41. Tayefi M, Shafiee M, Kazemi-Bajestani SMR, Esmaeili H, Darroudi S, Khakpouri S, et al. Depression and anxiety both associate with serum level of hs-CRP: A gender-stratified analysis in a population-based study. Psychoneuroendocrinology. 2017;81:63-9.
42. Liukkonen T, Silvennoinen-Kassinen S, Jokelainen J, Rasanen P, Leinonen M, Meyer-Rochow VB, et al. The association between C-reactive protein levels and depression: Results from the northern Finland 1966 birth cohort study. Biological psychiatry. 2006;60(8):825-30.
43. Li Y, Zhong X, Cheng G, Zhao C, Zhang L, Hong Y, et al. Hs-CRP and all-cause, cardiovascular, and cancer mortality risk: A meta-analysis. Atherosclerosis. 2017;259:75-82.
44. Lahti-Pulkkinen M, Girchenko P, Robinson R, Soili M. Lehto SM, Toffol E, Heinonen K, et al. Maternal depression and inflammation during pregnancy. Psychological medicine. 2019.
45. Girchenko P, Lahti M, Tuovinen S, Savolainen K, Lahti J, Binder EB, et al. Cohort Profile: Prediction and prevention of preeclampsia and intrauterine growth restriction (PREDO) study. International journal of epidemiology. 2017;46(5):1380-1g.
46. Soininen P, Kangas AJ, Wurtz P, Suna T, Ala-Korpela M. Quantitative serum nuclear magnetic resonance metabolomics in cardiovascular epidemiology and genetics. Circulation Cardiovascular genetics. 2015;8(1):192-206.
47. Sund R. Quality of the Finnish Hospital Discharge Register: a systematic review. Scandinavian journal of public health. 2012;40(6):505-15.
48. Squires J, Bricker D, Potter L. Revision of a parent-completed development screening tool: Ages and Stages Questionnaires. Journal of pediatric psychology. 1997;22(3):313-28.
49. Bricker D, Squires J, Kaminski R, Mounts L. The validity, reliability, and cost of a parent-completed questionnaire system to evaluate at-risk infants. Journal of pediatric psychology. 1988;13(1):55-68.
50. Kerstjens JM, Bos AF, ten Vergert EM, de Meer G, Butcher PR, Reijneveld SA. Support for the global feasibility of the Ages and Stages Questionnaire as developmental screener. Early human development. 2009;85(7):443-7.
51. Gwet KJG, MD: STATAXIS Publishing Company. Handbook of inter-rater reliability: How to estimate the level of agreement between two or multiple raters. 2001.
52. Arnson Y, Shoenfeld Y, Amital H. Effects of tobacco smoke on immunity, inflammation and autoimmunity. Journal of Autoimmunity. 2010;34(3):J258-J65.
53. Finy MS, Christian LM. Pathways linking childhood abuse history and current socioeconomic status to inflammation during pregnancy. Brain, behavior, and immunity. 2018;74:231-40.
54. Ronfani L, Vecchi Brumatti L, Mariuz M, Tognin V, Bin M, Ferluga V, et al. The Complex Interaction between Home Environment, Socioeconomic Status, Maternal IQ and Early Child Neurocognitive Development: A Multivariate Analysis of Data Collected in a Newborn Cohort Study. PloS one. 2015;10(5):e0127052.
55. Streissguth AP, Barr HM, Sampson PD, Darby BL, Martin DC. IQ at age 4 in relation to maternal alcohol use and smoking during pregnancy. Developmental psychology. 1989;25(1):3-11.
56. Kongsted A, Nielsen AM. Latent Class Analysis in health research. Journal of Physiotherapy. 2017;63(1):55-8.
57. Hayes AF, Rockwood NJ. Regression-based statistical mediation and moderation analysis in clinical research: Observations, recommendations, and implementation. Behaviour research and therapy. 2016.
58. Kohler O, Petersen L, Mors O, Mortensen PB, Yolken RH, Gasse C, et al. Infections and exposure to anti-infective agents and the risk of severe mental disorders: a nationwide study. Acta psychiatrica Scandinavica. 2017;135(2):97-105.

59. Lund-Sorensen H, Benros ME, Madsen T, Sorensen HJ, Eaton WW, Postolache TT, et al. A Nationwide Cohort Study of the Association Between Hospitalization With Infection and Risk of Death by Suicide. JAMA psychiatry. 2016;73(9):912-9.
60. Bánhidy F, Ács N, Puhó EH, Czeizel AE. Iron deficiency anemia: Pregnancy outcomes with or without iron supplementation. Nutrition (Burbank, Los Angeles County, Calif). 2011;27(1):65-72.
61. Lin P-Y, Chang C-H, Chong MF-F, Chen H, Su K-P. Polyunsaturated Fatty Acids in Perinatal Depression: A Systematic Review and Meta-analysis. Biological psychiatry. 2017;82(8):560-9.
62. Rios AC, Maurya PK, Pedrini M, Zeni-Graiff M, Asevedo E, Mansur RB, et al. Microbiota abnormalities and the therapeutic potential of probiotics in the treatment of mood disorders. Reviews in the neurosciences. 2017;28(7):739-49.
63. Luan W, Hammond LA, Vuillermot S, Meyer U, Eyles DW. Maternal Vitamin D Prevents Abnormal Dopaminergic Development and Function in a Mouse Model of Prenatal Immune Activation. Scientific reports. 2018;8(1):9741.
64. Vuillermot S, Luan W, Meyer U, Eyles D. Vitamin D treatment during pregnancy prevents autism-related phenotypes in a mouse model of maternal immune activation. Molecular autism. 2017;8:9.
65. Cesar HC, Pisani LP. Fatty-acid-mediated hypothalamic inflammation and epigenetic programming. The Journal of nutritional biochemistry. 2017;42:1-6.
66. Ma M, Ren Q, Yang J, Zhang K, Xiong Z, Ishima T, et al. Key role of soluble epoxide hydrolase in the neurodevelopmental disorders of offspring after maternal immune activation. Proceedings of the National Academy of Sciences of the United States of America. 2019;116(14):7083-8.
67. Akkasheh G, Kashani-Poor Z, Tajabadi-Ebrahimi M, Jafari P, Akbari H, Taghizadeh M, et al. Clinical and metabolic response to probiotic administration in patients with major depressive disorder: A randomized, double-blind, placebo-controlled trial. Nutrition (Burbank, Los Angeles County, Calif). 2016;32(3):315-20.
68. Koren O, Goodrich JK, Cullender TC, Spor A, Laitinen K, Backhed HK, et al. Host remodeling of the gut microbiome and metabolic changes during pregnancy. Cell. 2012;150(3):470-80.
69. Grosso G, Galvano F, Marventano S, Malaguarnera M, Bucolo C, Drago F, et al. Omega-3 fatty acids and depression: scientific evidence and biological mechanisms. Oxidative medicine and cellular longevity. 2014;2014:313570.
70. Caesar R, Tremaroli V, Kovatcheva-Datchary P, Cani PD, Backhed F. Crosstalk between Gut Microbiota and Dietary Lipids Aggravates WAT Inflammation through TLR Signaling. Cell metabolism. 2015;22(4):658-68.
71. Wirtz DC, Heller KD, Miltner O, Zilkens KW, Wolff JM. Interleukin-6: a potential inflammatory marker after total joint replacement. International orthopaedics. 2000;24(4):194-6.
72. Shaw P, Gogtay N, Rapoport J. Childhood psychiatric disorders as anomalies in neurodevelopmental trajectories. Human brain mapping. 2010;31(6):917-25.
73. Reef J, Diamantopoulou S, van Meurs I, Verhulst FC, van der Ende JJSP, Epidemiology P. Developmental trajectories of child to adolescent externalizing behavior and adult DSM-IV disorder: results of a 24-year longitudinal study. 2011;46(12):1233-41.
74. Childhood Origins Of Adult Health: A Basis For Life-Course Health Policy. 2004;23(5):155-64.
75. Seltzer MM, Shattuck P, Abbeduto L, Greenberg JS. Trajectory of development in adolescents and adults with autism. 2004;10(4):234-47.
76. Al-Haddad BJS, Jacobsson B, Chabra S, Modzelewska D, Olson EM, Bernier R, et al. Long-term Risk of Neuropsychiatric Disease After Exposure to Infection In Utero. JAMA psychiatry. 2019.
77. Atladottir HO, Thorsen P, Ostergaard L, Schendel DE, Lemcke S, Abdallah M, et al. Maternal infection requiring hospitalization during pregnancy and autism spectrum disorders. Journal of autism and developmental disorders. 2010;40(12):1423-30.
78. Lee BK, Magnusson C, Gardner RM, Blomström Å, Newschaffer CJ, Burstyn I, et al. Maternal hospitalization with infection during pregnancy and risk of autism spectrum disorders. Brain, behavior, and immunity. 2015;44:100-5.

79. Gumusoglu SB, Stevens HE. Maternal Inflammation and Neurodevelopmental Programming: A Review of Preclinical Outcomes and Implications for Translational Psychiatry. Biological psychiatry. 2019;85(2):107-21.
80. Paolicelli RC, Bolasco G, Pagani F, Maggi L, Scianni M, Panzanelli P, et al. Synaptic pruning by microglia is necessary for normal brain development. Science (New York, NY). 2011;333(6048):1456-8.
81. Frick LR, Williams K, Pittenger C. Microglial dysregulation in psychiatric disease. Clinical & developmental immunology. 2013;2013:608654.
82. Coiro P, Padmashri R, Suresh A, Spartz E, Pendyala G, Chou S, et al. Impaired synaptic development in a maternal immune activation mouse model of neurodevelopmental disorders. Brain, behavior, and immunity. 2015;50:249-58.

Figure legends

Figure 1. Box plots with medians (horizontal lines within boxes), interquartile ranges (error bars) and means (diamonds) of high sensitivity C-reactive protein (Panel A) and glycoprotein acetyls (Panel B) within the three subgroups of women with low, moderate and high level of inflammation at three consecutive antenatal blood sampling points. The three subgroups were identified as the most optimal solution by the Latent Class Analysis (LCA).

Figure 2. Mediation analysis showing that the effect of prenatal environmental adversity (overweight / obesity, diabetes, hypertensive disorders, mood or anxiety disorders: 0=no adversity, 1=any adversity) on the number of child neurodevelopmental delay areas across cognitive, motor and social skills (0=no delay in any area, 1=one delay in any area, 2=two delays in any area, 3=delay in all three areas) in a follow-up of 10.8 years is partially mediated via maternal inflammation (low, moderate and high level of high-sensitivity C-reactive protein and glycoprotein acetyls across the three timepoints during pregnancy identified by the latent class analysis) during pregnancy. Numbers above dashed lines show bi-variate associations between the variables, and those above solid lines results from the mediation analysis.

Supplemental Figure 1. Agreement between the any neurodevelopmental delay identified by the mother-reported Ages and Stages Questionnaire (ASQ) (≤-2SD for age on any ASQ domain) and any psychological development disorder diagnoses (International Classification of Disease Tenth Revision diagnostic codes F80-F84) identified by the Care Register for Health Care (HILMO) in the

clinical subsample (n=502) and the analytic sample of the current study (Panel B). AC1 refers to chance-corrected first-order agreement coefficient and 95% CI to 95% Confidence Interval.

Figure 1.

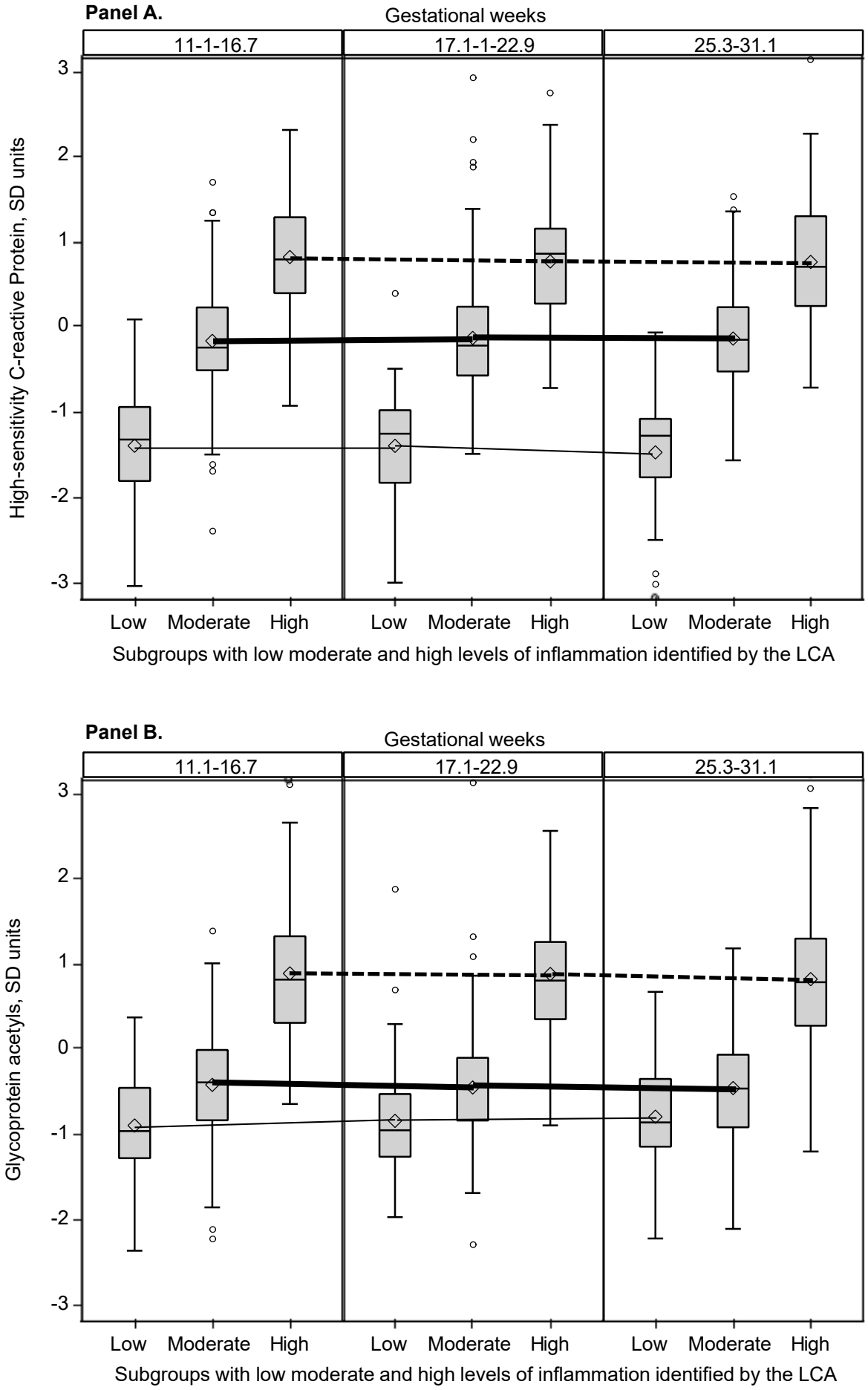
Panel A.
Gestational weeks
11-1-16.7
17.1-1-22.9
25.3-31.1
High-sensitivity C-reactive Protein, SD units
3
2
1
0
-1
-2
-3
Low
Moderate
High
Subgroups with low moderate and high levels of inflammation identified by the LCA
Panel B.
Gestational weeks
11.1-16.7
17.1-22.9
25.3-31.1
Glycoprotein acetyls, SD units
Subgroups with low moderate and high levels of inflammation identified by the LCA
High inflammation
Moderate inflammation
Low inflammation

Figure 2.

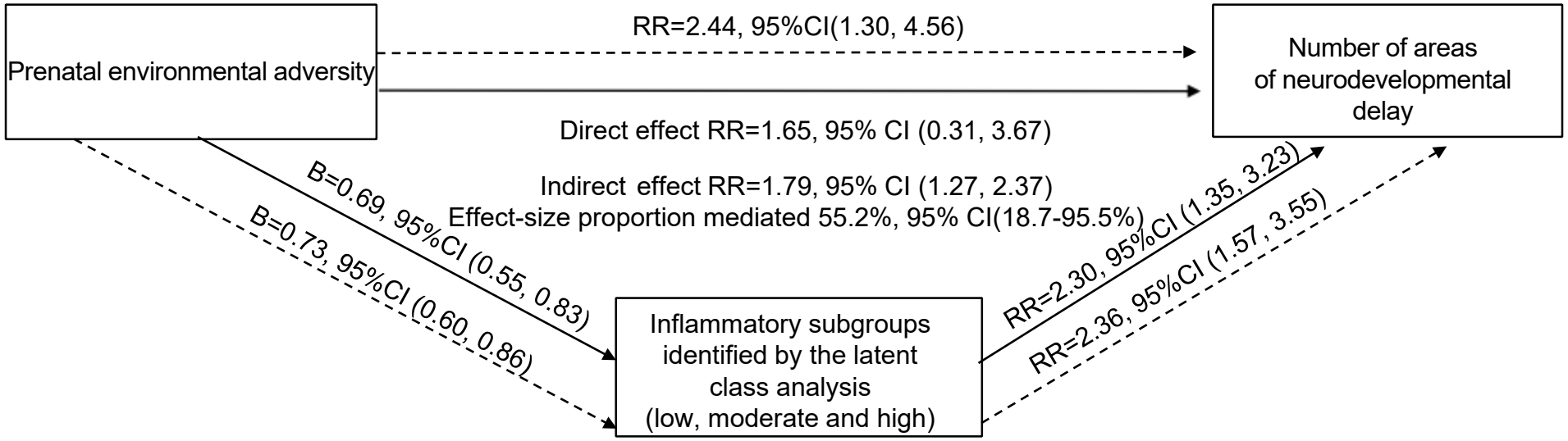
Prenatal environmental adversity
RR=2.44, 95%CI(1.30, 4.56)
Number of areas of neurodevelopmental delay
Direct effect RR=1.65, 95% CI (0.31, 3.67)
Indirect effect RR=1.79, 95% CI (1.27, 2.37)
Effect-size proportion mediated 55.2%, 95% CI(18.7-95.5%)
B=0.69, 95%CI (0.55, 0.83)
B=0.73, 95%CI (0.60, 0.86)
RR=2.30, 95%CI (1.35, 3.23)
RR=2.36, 95%CI (1.57, 3.55)
Inflammatory subgroups identified by the latent class analysis (low, moderate and high)

Table 1. Characteristics of the sample (418 mother-child dyads)

	Mean (SD) / N (%)	Range
Maternal characteristics		
Age at delivery (years)	32.6 (5.2)	19.0-47.4
< 40 years at delivery	375 (89.7%)	
≥ 40 years at delivery,	43 (10.3%)	
Missing	0	
Education		
Lower secondary or lower	201 (48.1%)	
Upper secondary or tertiary	211 (50.5%)	
Missing	6 (1.4%)	
Occupational status		
Manual laborer	68 (16.3%)	
Junior or senior clerical	85 (20.3%)	
Entrepreneur	87 (20.8%)	
Other or missing	178 (42.6%)	
Body Mass Index in early pregnancy(kg/m^2)	27.0 (6.5)	17.6-55.0
Normal weight (<24.99 kg/m^2)	209 (50.0%)	
Overweight (25-29.99 kg/m^2)	86 (20.6%)	
Obese (≥30 kg/m^2)	123 (29.4%)	
Missing	0	
Hypertensive disorders		
Normotension	266 (63.6%)	
Gestational hypertension	38 (9.1%)	
Pre-eclampsia	43 (10.3%)	
Chronic hypertension	71 (17.0%)	
Missing	0	
Diabetes disorders		
No	317 (75.8%)	
Gestational diabetes	92 (22.0%)	
Type 1 diabetes	9 (2.1%)	
Missing	0	
Any mood disorder diagnosed before delivery (F3)*		
No	398 (95.2%)	
Yes	20 (4.8%)	
Any anxiety/neurotic disorder diagnosed before delivery (F4)*		
No	400 (95.7%)	
Yes	18 (4.3%)	
Any prenatal environmental adversity		
No	135 (32.3%)	
Yes	283 (67.7%)	
Missing	0	
High-sensitivity C-reactive protein (mg/L)		
First blood sampling point (11.1-15.1 gestational weeks)	3.81 (5.16)	0.23-32.70
Missing	10 (2.4%)	
Second blood sampling point (17.1-22.9 gestational weeks)	4.53 (6.27)	0.31-60.65
Missing	15 (3.6%)	
Third blood sampling point (25.3-30.6 gestational weeks)	3.95 (4.80)	0.19-61.07
Missing	18 (4.3%)	

Glycoprotein acetyls (mmol/L)		
First blood sampling point (11.1-15.1 gestational weeks)	1.26 (0.16)	0.89-1.85
Missing	21 (5.0%)	
Second blood sampling point (17.1-22.9 gestational weeks)	1.35 (0.18)	0.94-2.14
Missing	20 (4.8%)	
Third blood sampling point (25.3-30.6 gestational weeks)	1.45 (0.18)	1.06-2.25
Missing	29 (6.9%)	
Child characteristics		
Sex		
Boy	223 (53.4%)	
Girl	195 (46.7%)	
Missing	0	
Year of birth		
2006	69 (16.5%)	
2007	99 (23.7%)	
2008	112 (26.8%)	
2009	138 (33.0%)	
Missing	0	
Disorders of psychological development (F8)*	33 (7.9%)	
Speech and language development (F80)*	20 (4.8%)	
Scholastic skills (F81)*	3 (0.7%)	
Motor development (F82)*	13 (3.1%)	
Mixed specific developmental disorders (F83)*	7 (1.7%)	
Autism spectrum disorder (F84)*	4 (1.0%)	
Missing	0	
Delay in achieving developmental milestone appropriate the child's age in the Ages and Stages Questionnaire (ASQ)		
Communication skills (≤ -2SD)	6 (1.4%)	
Fine motor skills (≤ -2SD)	8 (1.9%)	
Gross motor skills (≤ -2SD)	8 (1.9%)	
Problem solving skills (≤ -2SD)	8 (1.9%)	
Personal social skills (≤ -2SD)	8 (1.9%)	
Missing	223 (53.3%)	
Neurodevelopmental delay in the three broad areas		
Cognitive development (F80, F81, F83 or ≤ -2SD ASQ communication or problem solving skills)*	34 (8.1%)	
Motor development (F82 or ≤ -2SD ASQ fine or gross motor skills)*	25 (6.0%)	
Social development (F84 or ≤ -2SD ASQ personal social skills)*	12 (2.9%)	
Missing	0	
Number of neurodevelopmental delay across the three broad areas		
Delay in any of the areas	29 (6.9%)	
Delay in any two of the areas	15 (3.6%)	
Delay in all three areas	4 (1.0%)	
No delay in any area	370 (88.5%)	
Missing	0	

*International Classification of Diseases Tenth revision diagnostic codes

Table 2. Associations between maternal antenatal levels of high-sensitivity C-Reactive Protein and Glycoprotein acetyls and the number of areas of child neurodevelopmental delay across cognitive, motor and social development in a follow-up of up to 10.8 years

	Number of areas of neurodevelopmental delay (0=no delay in any area, 1=one delay in any area, 2=two delays in any areas, 3=delay in all three areas)		
	Relative Risk	95% Confidence Interval	P
High-sensitive C-Reactive Protein, SD units			
First blood sampling (11.1-16.7 gestational weeks)			
Model 1	1.50	1.16, 1.93	0.002
Model 2	1.56	1.19, 2.03	0.001
Model 3	1.41	1.07, 1.87	0.01
Second blood sampling (17.1-22.9 gestational weeks)			
Model 1	1.45	1.13, 1.86	0.004
Model 2	1.45	1.12, 1.87	0.005
Model 3	1.33	1.01, 1.75	0.04
Third blood sampling (25.3-31.1 gestational weeks)			
Model 1	1.72	1.34, 2.21	<0.0001
Model 2	1.76	1.36, 2.28	<0.0001
Model 3	1.64	1.25, 2.16	0.0004
Glycoprotein acetyls, SD units			
First blood sampling (11.1-16.7 gestational weeks)			
Model 1	1.36	1.08, 1.72	0.009
Model 2	1.42	1.10, 1.83	0.007
Model 3	1.26	0.96, 1.66	0.09
Second blood sampling (17.1-22.9 gestational weeks)			
Model 1	1.30	1.05, 1.61	0.02
Model 2	1.25	1.00, 1.58	0.05
Model 3	1.15	0.90, 1.47	0.26
Third blood sampling (25.3-31.1 gestational weeks)			
Model 1	1.46	1.17, 1.81	0.0006
Model 2	1.50	1.19, 1.88	0.0006
Model 3	1.39	1.09, 1.79	0.009

Note. Model 1 is adjusted for child sex and birth year, Model 2 is additionally adjusted for maternal age at delivery, education, occupation and smoking and alcohol use during pregnancy, Model 3 is additionally adjusted for prenatal adversity (maternal overweight/obesity, diabetes and hypertensive, mood and anxiety disorders).

Supplemental Table 1. Comorbidity of prenatal environmental adversities in women with any prenatal environmental adversity.

Number of prenatal environmental adversities	Overweight/Obesity	Hypertensive disorders	Diabetes disorders	Mood disorders	Anxiety disorders	Number of women with any prenatal environmental adversity
One prenatal environmental adversity						**126**
	X					64
		X				37
			X			21
				X		1
					X	3
Two prenatal environmental adversities						**107**
	X	X				64
	X		X			27
	X			X		3
	X				X	1
		X	X			7
		X			X	2
			X	X		1
				X	X	2
Three prenatal environmental adversities						**42**
	X	X	X			36
	X		X	X		2
	X			X	X	4
Four prenatal environmental adversities						**6**
	X	X	X	X		2
	X	X	X		X	1
	X		X	X	X	2
	X	X		X	X	1
Five prenatal environmental adversities						**2**
	X	X	X	X	X	2
Total	**209**	**152**	**101**	**20**	**18**	**283**

Supplemental Table 2. Associations between maternal antenatal levels of high-sensitivity C-Reactive Protein and glycoprotein acetyls and specific neurodevelopmental delay areas.

	Cognitive delay			Motor delay			Social delay		
	Odds Ratio	95% Confidence Interval	P	Odds Ratio	95% Confidence Interval	P	Odds Ratio	95% Confidence Interval	P
High-sensitivity C-Reactive Protein, SD units									
First blood sampling (11.1-16.7 gestational weeks)									
Model 1	1.35	0.92, 1.98	0.13	1.64	1.05, 2.58	0.03	2.08	1.07, 4.04	0.03
Model 2	1.31	0.87, 1.96	0.20	1.81	1.11, 2.94	0.02	2.35	1.17, 4.72	0.02
Model 3	1.14	0.74, 1.76	0.54	1.63	0.98, 2.70	0.06	2.23	1.08, 4.59	0.03
Second blood sampling (17.1-22.9 gestational weeks)									
Model 1	1.23	0.84, 1.80	0.30	1.88	1.20, 2.96	0.006	1.60	0.88, 2.93	0.13
Model 2	1.15	0.77, 1.72	0.50	2.01	1.25, 3.22	0.004	1.75	0.94, 3.27	0.08
Model 3	1.00	0.65, 1.55	0.99	1.88	1.14, 3.11	0.01	1.65	0.85, 3.20	0.14
Third blood sampling (25.3-31.1 gestational weeks)									
Model 1	1.68	1.12, 2.50	0.01	1.86	1.17, 2.95	0.008	2.20	1.17, 4.14	0.01
Model 2	1.64	1.08, 2.48	0.02	1.93	1.20, 3.11	0.007	2.27	1.20, 4.28	0.01
Model 3	1.52	0.98, 2.37	0.06	1.73	1.05, 2.85	0.03	2.16	1.08, 4.31	0.03
Glycoprotein acetyls, SD units									
First blood sampling (11.1-16.7 gestational weeks)									
Model 1	1.28	0.88, 1.84	0.19	1.69	1.13, 2.53	0.01	1.24	0.68, 2.25	0.48
Model 2	1.25	0.85, 1.85	0.25	1.79	1.17, 2.74	0.008	1.30	0.70, 2.42	0.41
Model 3	1.09	0.72, 1.66	0.68	1.62	1.03, 2.54	0.04	1.15	0.58, 2.25	0.69
Second blood sampling (17.1-22.9 gestational weeks)									
Model 1	1.18	0.82, 1.68	0.38	1.65	1.14, 2.38	0.008	1.17	0.67, 2.07	0.58
Model 2	1.09	0.75, 1.58	0.65	1.64	1.12, 2.40	0.01	1.21	0.68, 2.18	0.52
Model 3	0.96	0.64, 1.44	0.84	1.53	1.02, 2.28	0.04	1.10	0.59, 2.07	0.76
Third blood sampling (25.3-31.1 gestational weeks)									
Model 1	1.54	1.08, 2.19	0.02	1.72	1.17, 2.54	0.006	1.20	0.67, 2.17	0.54
Model 2	1.52	1.06, 2.18	0.02	1.76	1.18, 2.63	0.006	1.23	0.69, 2.19	0.49
Model 3	1.44	0.97, 2.14	0.05	1.61	1.05, 2.49	0.03	1.07	0.56, 2.04	0.84

Note. Model 1 is adjusted for child sex and birth year, Model 2 is additionally adjusted for maternal age at delivery, education, occupation and smoking and alcohol use during pregnancy, Model 3 is additionally adjusted for prenatal adversity (maternal overweight/obesity, diabetes and hypertensive, mood and anxiety disorders).

Supplemental Table 3. Criteria used in Latent Class Analyses for identifying the most optimal solution to group women based on their high-sensitivity C-reactive protein and glycoprotein acetyls at three consecutive antenatal blood sampling points.

Criteria:	Number of groups based on the Latent Class Analyses				
	2	3	4	5	6
Goodness-of-fit					
Akaike Information Criterion	5936.75	5717.44	5593.29	5530.94	5458.79
Bayesian Information Criterion	6013.43	5822.67	5726.46	5692.36	5648.46
Number (%) of participants in groups					
1	217 (51.9%)	71 (17.0%)	65 (15.6%)	67 (16.0%)	2 (0.5%)
2	201 (48.1%)	181 (43.3%)	173 (41.4%)	159 (38.0%)	112 (28.1%)
3		166 (39.7%)	144 (34.5%)	86 (20.6%)	111 (27.9%)
4			36 (8.6%)	28 (6.7%)	80 (20.1%)
5				78 (18.7%)	25 (6.3%)
6					68 (17.1%)
Certainty of classification by posterior probability	0.96 (SD 0.10)	0.93 (SD 0.12)	0.91 (SD 0.12)	0.88 (SD 0.15)	0.86 (SD 0.15)
Clinical relevance	Clearly defined groups with consistently low and consistently high inflammation.	Clearly defined groups with consistently low, consistently moderate and consistently high inflammation.	Clearly defined groups with consistently low and consistently moderate inflammation, two groups with consistently high inflammation are not clearly different	Clearly defined group with consistently low inflammation, two groups with consistently moderate inflammation are not clearly different, two groups with consistently high inflammation are not clearly different	Two groups with consistently low inflammation are not clearly different, two groups with consistently moderate inflammation are not clearly different, two groups with consistently high inflammation are not clearly different
	Clinically relevant. Easy to identify women at risk	Clinically relevant. Easy to identify women at risk, more targeted identification of risk as compared to the two group solution	Clinical relevance of segregating the groups that do not distinctly differ from each other is not clear. LCA with 4-6 groups can be scaled down to the 3 groups solution.		

Supplemental Table 4. Criteria used in Latent Class Analyses for identifying the most optimal solution to group women based on their high-sensitivity C-reactive protein at three consecutive antenatal blood sampling points.					
Criteria:	Number of groups based on the Latent Class Analyses				
	2	3	4	5	6
Goodness-of-fit					
Akaike Information Criterion	3017.26	2834.08	2760.43	2722.72	2668.08
Bayesian Information Criterion	3057.61	2890.58	2833.07	2811.50	2773.00
Number (%) of participants in groups					
1	170 (40.7%)	56 (13.4%)	33 (7.9%)	10 (2.4%)	9 (2.2%)
2	248 (59.3%)	196 (46.9%)	126 (30.1%)	53 (12.7%)	54 (12.9%)
3		166 (39.7%)	177 (42.3%)	133 (31.82%)	129 (30.9%)
4			82 (19.6%)	150 (35.9%)	141 (33.7%)
5				72 (17.2%)	76 (18.2%)
6					9 (2.2%)
Certainty of classification by posterior probability	0.94 (SD 0.12)	0.91 (SD 0.13)	0.88 (SD 0.14)	0.87 (SD 0.14)	0.87 (SD 0.14)
Clinical relevance	Clearly defined groups with consistently low and consistently high inflammation.	Clearly defined groups with consistently low, consistently moderate and consistently high inflammation.	Two groups with consistently low inflammation are not clearly different, clearly defined groups with consistently moderate and consistently high inflammation	Three groups with consistently low inflammation are not clearly different, clearly defined groups with consistently moderate and consistently high inflammation	Three groups with consistently low inflammation are not clearly different, clearly defined groups with consistently moderate and high inflammation, group 6 (n=9) displayed low inflammation at the first and third time points and high inflammation at the second time point
	Clinically relevant. Easy to identify women at risk	Clinically relevant. Easy to identify women at risk, more targeted identification of risk as compared to the two group solution	Clinical relevance of segregating the groups that do not distinctly differ from each other is not clear. LCA with 4-6 groups can be scaled down to the 3 group solution.		

Supplemental Table 5. Sensitivity analyses of associations between maternal inflammatory biomarker subgroups identified by the latent class analysis (groups on low, moderate and high levels of high-sensitivity C-reactive protein and glycoprotein acetyls across the three consecutive antenatal timepoints) and the number of areas of child neurodevelopmental delay across cognitive, motor and social development in a follow-up to10.8 years

Low, moderate and high level of maternal inflammation based on Latent Class Analysis	Number of areas of neurodevelopmental delay (0=no delay in any area, 1=one delay in any area, 2=two delays in any areas, 3=delay in all three areas)		
	Relative Risk	95% Confidence Interval	P
Sensitivity analysis 1: women who were diagnosed with any mood or anxiety disorder after the delivery are excluded (n=34)	2.18	1.40, 3.39	0.0005
Sensitivity analysis 2: women who were diagnosed with acute infectious diseases, rheumatoid arthritis, asthma, herpes simplex are excluded (n=29)	2.19	1.45, 3.32	0.0002
Sensitivity analysis 3: women whose maximum hemoglobin value during pregnancy was lower than 105 g/L are excluded (n=79)	2.17	1.35, 3.48	0.001
Sensitivity analysis 4: mother-reported Ages and Stages Questionnaire data are excluded from the child's developmental delay variable (n=34 mother-reported delays; child's developmental delay is based only on the Care Register for Health Care disorder diagnosis data)	1.97	1.07, 3.60	0.03

Note. The models are adjusted for child sex and birth year.

Supplemental Figure1